Reducing Lipid Peroxidation Stress of Erythrocyte Membrane by α-Tocopherol Nicotinate Plays an Important Role in Improving Blood Rheological Properties in Type 2 Diabetic Patients with Retinopathy

T.-W. Chung*[1], J.J. Hau Yu[2] D.-Z. Liu[1]

[1]*Department of Biomedical Engineering, Chung-Yuan Christian University, Chung-Li, Taiwan, China*
[2]*Department of Ophthalmology, Taipei Municipal Chung-Hsiao Hospital, Taipei, Taiwan, China*

The effects of α-tocopherol nicotinate on blood viscoelasticity and viscosity and on lipid peroxidation stress in erythrocyte membranes in patients with Type 2 DM were investigated. Thirteen Type 2 diabetic subjects with retinopathy were given α-tocopherol nicotinate 300 mg tds, after meals, for 3 months. The treatment resulted in significant reductions of blood viscosity at different shear rates (e.g. -2.23 ± 2.82 $p<0.015$, $\dot{\gamma} = 1.5\ s^{-1}$) and viscoelasticity ($p<0.004$); resistance of erythrocyte deformation ($p<0.001$) and lipid peroxidation stress in red cell membrane (malondialdehyde or MDA reduced by 0.17 ± 0.13 nmol l^{-1} $p<0.005$). Plasma viscosity, red cell rigidity, and HbA_{Ic} were unchanged. There were negative linear correlations between the indices of red cell deformability and the levels of MDA of red cell membrane both pre- and post-treatment (e.g. $R = -0.79$, $p<0.001$; $R = -0.78$, $p<0.002$, $n = 13$; pre- and post-, respectively). We suggest that the improvements of rheological properties of blood and red cell deformability by α-tocopherol nicotinate are mainly attributed to reducing lipid peroxidation stress on membrane of red blood cells. The treatment may be useful in slowing deterioration of microangiopathy in Type 2 DM. © **1998 John Wiley & Sons, Ltd.**

Diabet. Med. **15: 380–385 (1998)**

KEY WORDS **α-tocopherol; haemorheology; lipid peroxidation; erythrocyte; Type 2 DM**

Received 21 May 1997; revised 4 December 1997; accepted 11 January 1998

Introduction

The abnormalities of blood rheology found in diabetic patients have usually been attributed to increased plasma and whole blood viscosity,[1,2] red cell rigidity (T_k)[3,4] and red cell aggregation,[5,6] and reduced deformability of red and white cells.[7,8] These abnormalities of blood rheological parameters may play a role in impaired blood flow in retinal capillaries[9,10] or in the peripheral circulation[11] of diabetic patients.

Chronic hyperglycaemia may result in increased lipid peroxidation in erythrocyte membrane,[12] and oxidation of membrane spectrin.[13] Oxidative stress might cause abnormalities of red cell membrane which alter the rheological properties of the cells and blood. On the other hand, we have suggested that oral administration of α-tocopherol nicotinate for 3 months can improve red cell deformability and reduced blood viscosity in high shear rates ($\dot{\gamma}$) in subjects with Type 2 DM and, consequently, may improve retinal capillary blood flow velocity.[14] This may be due to reduced oxidative stress of erythrocyte membrane. To investigate the mechanisms by which α-tocopherol nicotinate may improve blood rheological properties in Type 2 DM, we measured the level of lipid peroxidation stress of red cell membrane (by measuring malondialdehyde or MDA) and non-enzymatic glycosylation of haemoglobin (HbA_{1c}) before and after treatment. Moreover, the effects of the treatment on low shear rate viscosity and viscoelastic properties of blood were also studied.

Patients and Methods

Twenty Type 2 DM patients who had neither a history nor evidence of cardiovascular diseases (such as hypertension) but who did all have retinopathy were

* Correspondence to: Dr T.-W. Chung, Department of Biomedical Engineering, Chung-Yuan Christian University, Chung-Li, Taiwan 32023, China

 CCC 0742–3071/98/050380–06$17.50
© 1998 John Wiley & Sons, Ltd.

recruited. Retinopathy was diagnosed by ophthalmoscopic and fluorescent angiographic examination. Patients took no medication for 3 days prior to testing. Thirteen patients (8 F, 5 M) returned for the final evaluation. Their mean age was 62 (range 54–70) years with a mean duration of diabetes of 9 (range 6–13) years. Some of the patients tried to control the blood glucose level by medication but failed before they were involved in the study. Most patients were in poor glycaemic control, with a mean HbA_{1c} of $8.6 \pm 2.1\%$. Some had tried oral hypoglycaemic medication prior to study, but failed and no treatment was given to reduce blood glucose level after recruitment.

After giving informed consent, patients were fasted for 8 h prior to phlebotomy. Blood rheological properties and biochemical analysis were determined (see below) at baseline. Each patient then received α-tocopherol nicotinate (Eisai Co., Japan) by mouth for 3 months, in a dose of a 300 mg capsule three times a day after meals. The baseline measurements were repeated after 3 months of active treatment.

For comparison the degree of the possible improvements for those rheological properties of blood and biochemical parameters for the patients after the treatment, 8 (5 F, 3 M) age and weight-matched controls were recruited to test those properties. The protocols were approved by institutional human studied board.

Haematological and Rheological Measurements

After overnight fasting, venous blood was drawn from patients with EDTA as an anticoagulant (1.5 mg EDTA ml^{-1}). Blood cell counts and other haematological data such as mean cell volume (MCV) and haematocrit (Hct) were measured by an automatic cell counter (SYSMEX NE-800, TOA medical electronic Co., Kobe, Japan). Plasma was separated from blood by centrifugation at 1500 g for 5 min and its viscosity was measured by a capillary micro-viscometer (type 51610/I, Schott Gerated Ltd, Germany) at 37°C. Plasma fibrinogen and HbA_{IC} were determined by thrombin clot technique[15] and agar gel electrophoresis,[16] respectively.

Blood viscosity corrected for haematocrit at 40 ± 1 % was measured by a Hakke RS-100 double cone viscometer (HAAKE Mess-Technik, Karlsruhe, Germany), with a cone angle of 2°C at 37°C. The viscosity of blood for different shear rates was measured continuously by computer controlled tested programs. We report the data measured at shear rates of 400 s^{-1}, 40 s^{-1}, and 1.5 s^{-1}, reflecting high, medium, and low shear rates.

For testing the viscoelastic properties of blood at Hct of 40 %, we measured the samples in an oscillatory mode. For the sensitivity of testing, we measured the blood samples in a fixed frequency, $f = 0.22$ Hz, with different shear stress which ranged from 0.01 Pa to 0.3 Pa. The shear flow conditions correspond to the initial shear rates of 2 s^{-1} to 20 s^{-1}, respectively. Here, we report the data for dynamic viscosity and elasticity of blood measured at 2 s^{-1}.

The preparation of red cell suspensions for red cell deformability used constant flow rate filtration methods,[17,18] as previously reported,[14] and the components used for preparing the solutions for the red cell suspensions have also been published.[17,18] After preparation, 10 % Hct of red cell suspensions, in which white cell concentrations were usually less than 100 cells mm^{-3}, were filtered through 5 μm pore size Nuclepore membranes (Lot Number: AI54CX11A030, Poretecs, CA, USA) with a disc diameter of 13 mm and 0.8 cm^2 of effective area at a constant flow rate of 1.61 ml min^{-1}. The pressure–time data were measured with a pressure transducer (Model DP45, Validyne Engineering Corporation, Northridge, USA) connected to a Validyne digital transducer indicator (Model CD-23). The continuous output data of the indicator were digitized and recorded on an IBM PC/AT computer by a data acquisition/analysis system (S16–02, Coulbourn/DATAQ Instrument and W.C. Software Corporation, Akron, OH, USA) at a fixed sampling rate of 100 Hz. Recorded data were played back in off-line, and P_0 values for ringer solutions and P_i values for red cell suspensions were determined as reported.[17] β values were calculated by using the data of P_i/P_0 and indexed as resistance of red cells when flowing through the pores. The values of $1/\beta$ were selected as an index of red cell deformability. Red cell rigidity (T_k) was calculated at a shear rate of 400 s^{-1} by the equation of Dintenfass.[19]

To measure the oxidative stress of erythrocyte membranes, the product of lipid peroxidation, malondialdehyde (MDA), which reacts with thiobarbituric acid (TBA), was measured by determining the quantities of the MDA–TBA complex at 532 nm by a spectrophotometer (Hitachi U-2000, Hitachi Corporation, Japan).[20] The detailed preparation procedures for measuring the MDA-TBA complex have been described.[12] The quantities of MDA presented in results are based on 10^{10} erythrocytes.[12] Both biochemical analyses were determined in a blinded manner.

Data are presented as mean $\pm$ sd. All data were normally distributed; pre- and post-treatment data were compared by two-tailed, paired *t*-test and data from patients after treatment were compared with data from the non-diabetic control group by two tailed *t*-test. Linear regressions with higher than 95 % confidence level were also calculated. All calculations were analysed by SigmaStat Statistical software (Jandel Scientific, San Rafael, CA, USA) for IBM PC/AT.

Results

There were no significant changes ($P > 0.05$) for the patients in haemoglobin contents or haematocrit before and after 3 months of oral administration of α-tocopherol nicotinate (data not shown). Moreover, neither total proteins nor lipoproteins were significantly different after

© 1998 John Wiley & Sons, Ltd.

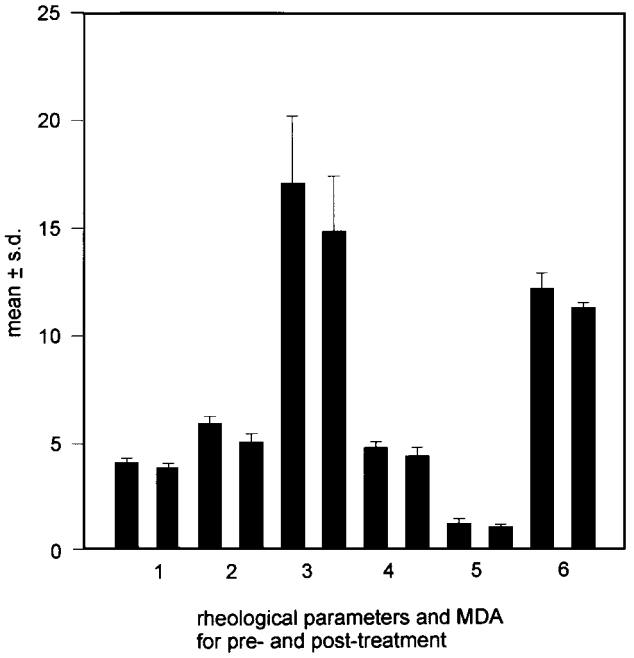

Figure 1. The mean values of rheological parameters of blood and MDA of red cell membrane for the patients before and after the treatment ($n = 13$). Note: η_{blood}: 1. $\dot{\gamma} = 400\ s^{-1}$, 2. $\dot{\gamma} = 40\ s^{-1}$, 3. $\dot{\gamma} = 1.5\ s^{-1}$, 4. β values, 5. MDA, 6. η'_{blood} ($\dot{\gamma} = 2\ s^{-1}$)

treatment. This is consistent with our previous reports.[14] HbA_{Ic} did not change significantly after treatment (before: 8.61 ± 2.08; after: 8.53 ± 2.52, $P > 0.05$, $n = 13$).

In contrast, blood viscosity was reduced in all tested shear rates corrected to a haematocit of 40 % after treatment. Here, we report the data of three different shear rates (Figure 1 and Table 1). However, there were no changes in plasma viscosity after treatment (Table 1). Blood viscoelasticity measured at a fixed frequency of 0.22 Hz was significantly improved in both dynamic viscosity (η') and elasticity (η'') at a corresponding shear rate (Figure 1 and Table 1) after treatment.

Table 1. The differences of haemorheological data for the pre- and post-treated Type 2 DM patients with α-tocopherol nicotinate ($n = 13$)

Parameters	Difference mean ± SD	Paired *t*-test
η_{plasma} (cP)	–0.01 ± 0.025	$p = 0.20$; NS
η_{blood} (cP)[a] ($\dot{\gamma} = 400\ s^{-1}$)	–0.25 ± 0.16	$p < 0.0014$
η_{blood} (cP) ($\dot{\gamma} = 40\ s^{-1}$)	–0.88 ± 0.42	$p < 0.0001$
η_{blood} (cP) ($\dot{\gamma} = 1.5\ s^{-1}$)	–2.23 ± 2.82	$p < 0.015$
η'_{blood} (cP)[a] ($\dot{\gamma} \approx 2\ s^{-1}$)	–0.91 ± 0.63	$p < 0.0004$
η''_{blood} (cP)[b] ($\dot{\gamma} \approx 2\ s^{-1}$)	–0.24 ± 0.25	$p < 0.004$

[a]The unit for blood viscosity is centi-poise(cP).
[b]η' and η'' are dynamic viscosity and elasticity of blood.

Table 2. The differences of erythrocyte rheological and biochemical properties for the pre- and post-treated Type 2 DM ($n = 13$)

Parameter	Difference mean ± SD	Paired *t*-test
T_k ($\dot{\gamma} = 400\ s^{-1}$)[a]	–0.0031 ± 0.0066	P = 0.12; NS
β-values	–0.41 ± 0.27	P = 0.00016
MDA (nmol l^{-1})[b]	–0.17 ± 0.13	P = 0.0005
HbA_{Ic}(%)	–0.085 ± 1.25	P = 0.81; NS

[a]Definition: $T_k = (\eta_r^{0.4} - 1)/(\eta_r^{0.4} \times \text{Hct})$
The data were tested at $\dot{\gamma} = 400\ s^{-1}$.
[b]The quantity of MDA is based on 10^{10} erythrocytes.

Red cell deformability, an index of the rheological properties of red cells, is usually expressed as a β value which is lower (or the reciprocal of β which is higher). The deformability of red cells was significantly improved ($p < 0.0016$, Figure 1 and Table 2) after treatment. However, there was no change in red cell rigidity (Table 2), which indicated that the changes for the viscosity of haemoglobin inside erythrocytes was less important in affecting the deformability of red cells after treatment.

There was a significant reduction in MDA of red cell membrane ($p < 0.005$) after treatment (Figure 1 and Table 2). We correlated the red cell deformability index, $1/\beta$, with the values of MDA for both pre- and post-treated conditions. There were inverse correlations between $1/\beta$ and MDA values at both times ($r = -0.793$, $p < 0.001$; $R = -0.779$, $p < 0.002$, $n = 13$; pre- and post-, respectively). The correlation after treatment is shown in Figure 2. The higher the MDA contents of red cell membranes, the lower the red cell deformability index.

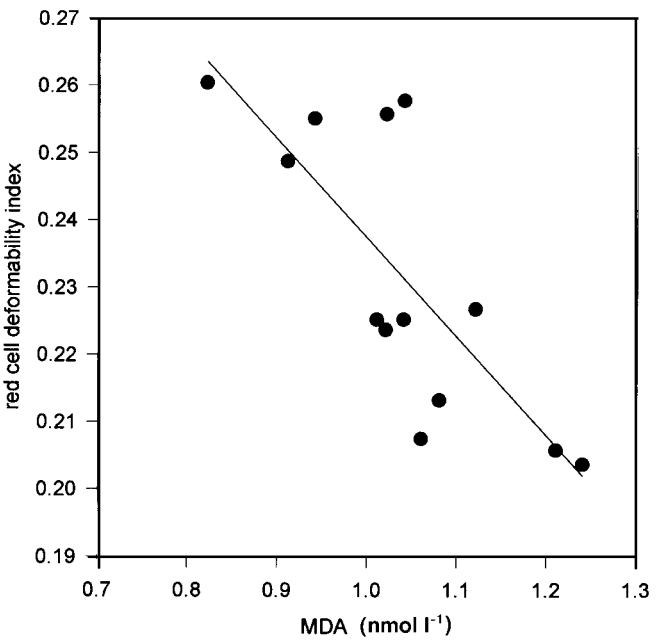

Figure 2. Linear regression for the deformability index ($1/\beta$) and MDA of erythrocyte membrane for the patients after treatment ($R = -0.78$, $p < 0.002$, $n = 13$)

© 1998 John Wiley & Sons, Ltd.

All parameters measured were significantly higher in diabetic patients after treatment when compared with non-diabetic controls (Table 3).

Discussion

Impairment of the rheological properties of blood has been well documented in diabetes.[1–10] We have reported impaired plasma viscosity and red cell deformability in Type 2 diabetic patients with background retinopathy and suggested this might result in decreased retinal capillary blood flow velocity.[9,14] Other investigators also reported abnormalities in erythrocyte membrane properties of diabetic patients or rats partially attributable to lipid peroxidation stress,[12] oxidation of spectrin[13] or reduced vitamin E content of cell membrane.[21] We and other investigators[14,22,23] have reported that supplementation with α-tocopherol related drugs can improve erythrocyte deformability and micro-viscosity of red cell membrane for diabetic subjects and rats, respectively. However, the effects of α-tocopherol nicotinate on blood rheological properties, especially blood viscosity in low shear rates and viscoelasticity of blood, and the possible relations of those properties with lipid peroxidation stress of red cells have not been well studied and the ability of such treatments to restore rheological parameters to normal is not known.

We found no significant change in plasma viscosity after α-tocopherol treatment in Type 2 DM, consistent with our previous report.[14] This is probably because there were no significant effects of α-tocopherol nicotinate on the concentrations of fibrinogen and albumin (data not shown), although vitamin E concentration in plasma may increase.[24] In contrast, steady decrease in blood viscosity at different ranges of shear rates were observed after treatment. Since there were no changes in plasma viscosity and red cell rigidity, improvement of erythrocyte membrane properties must be the main contributor to reduced blood viscosity at different ranges of shear rates, especially at high shear rates.

Table 3. Comparisons of rheological parameters and MDA for the post-treated Type 2 DM with those values for the non-diabetic control group

Parameters	Non-diabetic ($n=8$) mean ± SD	Type 2 DM ($n=13$) mean ± SD	*t*-test
η_{blood} (cP)[a] ($\gamma = 400\ s^{-1}$)	3.60 ± 0.07	3.88 ± 0.21	$p<0.03$
η_{blood} (cP) ($\gamma = 40\ s^{-1}$)	4.30 ± 0.12	4.98 ± 0.40	$p<0.01$
η_{blood} (cP) ($\gamma = 1.5\ s^{-1}$)	12.30 ± 0.8	14.80 ± 2.54	$p<0.01$
η'_{blood} (cP)[b] ($\gamma \approx 2\ s^{-1}$)	8.21 ± 0.40	11.15 ± 0.25	$p<0.001$
η''_{blood} (cP)[b] ($\gamma \approx 2\ s^{-1}$)	2.53 ± 0.08	3.31 ± 0.08	$p<0.01$
MDA(nmole)	0.79 ± 0.05	1.03 ± 0.11	$p<0.01$

[a]The unit for blood viscosity is centi-poise (cP).
[b]η' and η'' are dynamic viscosity and elasticity of blood.

Viscoelasticity of blood has been studied before.[25–27] However, the viscoelastic properties of blood have not been widely investigated and applied in clinical studies. Viscoelasticity of blood is primarily determined by the aggregation and disaggregation of red blood cells at low shear rates. The parameters that characterize the viscoelastic properties of blood are η' and η'', dynamic viscosity and elasticity of blood, respectively. The factors which would affect the properties of those parameters are dependent on haematocrit, shear stress, and frequency of oscillating shear flow applied to the samples. In general, η' and η'' reflect the ability of red cell aggregates to adjust their shape in low shear flow state and the elastic properties of red cell aggregates, respectively. It is possible to obtain qualitative information of blood when it flows in large vessels in pulsation,[28] and of 'rouleaux' formation in microcirculation. Values of η' and η'' have been reported to be significantly higher in the blood of diabetic patients with retinopathy than in non-diabetic subjects.[25] Moreover there was a significant correlation between blood viscosity of low shear rate (e.g. $1\ s^{-1}$) and η'' value for studied subjects.[25]

In this study, the lower values of η' and η'' of blood after α-tocopherol treatment of patients with Type 2 DM may suggest that the treatment can either enhance the ability of red cell aggregates to adjust themselves or diminish the structures of red cell agglomerates in shear flow. The improvements of η' and η'' of blood should therefore enhance blood flow in different sizes of blood vessels.

To investigate the effects of treatment on the deformation of red cells, we tested the deformability of erythrocytes by a constant flow filtration method,[17,18] expressing the results as β values.[17] The effect of residual white blood cells in the erythrocyte suspensions was minimized during filtration by using a constant flow method.[17,18] In this study, we found that α-tocopherol treatment resulted in lower values of β (or higher values of $1/\beta$) which indicates improvements of red cell deformability. This could play a major role in reducing blood viscosity tested at high shear rates (e.g. $400\ s^{-1}$), since red cell rigidity of blood was not changed after the treatment. Furthermore, it may be also a factor to reduce blood viscosity measured at low shear rates (e.g. $1.5\ s^{-1}$) after the treatment since plasma viscosity and the concentration of fibrinogen were not changed.

The product of lipid peroxidation of erythrocyte membrane, MDA, was reduced post-treatment. Measuring MDA by TBARS to quantify the stress of lipid peroxidation is a sensitive, simple, and widely used method, although we also need to be aware that MDA and other TBA reactants may also be formed from other products of polyunsaturated fatty acids as lipid hydroperoxides during the assay.[12]

It has been reported that higher HbA_{Ic} may increase the quantities of phospholipid-MDA adduct, and the

© 1998 John Wiley & Sons, Ltd.

values of HbA_{1c} level were inversely correlated with red cell deformability measured by an ektacytometer in diabetic patients.[13] However, there were no significant changes in HbA_{1c} after treatment, consistent with another report.[24] It is suggested that changes in HbA_{1c} play a very minor role in the rheological properties of erythrocytes. In contrast, treatment did reduce the quantities of MDA, indicating that the oxidative stress of red cell membrane was reduced. With the results of reducing MDA and increasing $1/\beta$ of red cell membrane for patients after the treatment, we postulated that reducing the level of MDA was the main factor improving deformability of red cells, and this hypothesis is supported by the inverse correlation between the deformability index (i.e. $1/\beta$) and the quantities of MDA of red cells after the treatment. Such a relationship was also found before treatment ($R = -0.79$, $p < 0.001$, $n = 13$). It is also suggested that reducing the oxidative stress of red cell membrane may enhance the deformability of red cells. Therefore, we suggested that reducing peroxidation stress of red cell membrane contributes to enhance the deformability of erythrocytes and then reduce the viscosity of blood for the patients after the treatment. Moreover, the reduced oxidative stress might also affect cell membranes in other tissues.

However, the values of viscosity and viscoelasticity of blood and the quantities of MDA of red cell membrane for diabetic patients of post-treated remained higher than those values of non-diabetic control subjects. Other factors such as high blood glucose level may still cause the abnormality of rheological properties and oxidation stress of red cell membrane for diabetic patients.

The rheological behaviour of blood was strongly dependent on the ability of red cells or red cell agglomerates to adapt themselves to shear flow field and the elasticity of red cell agglomerates. The improvement of red cell membrane deformability, due to reduced lipid peroxidation stress, may contribute to lowering the viscosity of blood at different shear rates, and viscoelastic properties of blood for patients after α-tocopherol treatment. We cannot rule out the effect of glycation of albumin on erythrocyte aggregations[6] at low shear rates state. However, neither the HbA_{1c} level, total plasma protein level, nor plasma viscosity were significantly changed, so plasma proteins and HbA_{1c} level must play a minor role in improving those rheological properties.

We conclude that 3 months of oral administration of α-tocopherol nicotinate for the Type 2 DM patients with retinopathy can reduce blood viscosity for different shear rates, reduce viscoelasticity of blood, and improve deformability of red cells. The improvements of those parameters after the treatment are most likely attributed to reduced lipid peroxidation stress of erythrocytes. However, the rheological properties and the oxidative stress of red cell membrane in the diabetic patients was not restored to normal. However, while the treatment may, at least in theory, be beneficial for Type 2 DM patients in slowing the deterioration of microangiopathy, control of blood glucose is still an important issue! The ability of these improvements in rheology to affect the course of diabetic retinopathy in the long term now needs to be investigated.

Acknowledgements

We would like to thank the staff of the Clinical Department, Taeipei Municipal Chung-Hsiao Hospital for their technical assistance. Y.L. Chang is also acknowledged for help with measurements. The project is supported by the National Science Council of China (project no. NSC-85-2214-E-033-001).

References

1. Hoare EM, Barnes AJ, Dormandy AJ. Abnormal blood viscosity in diabetes mellitus and retinopathy. *Biorheology* 1976; **13**: 21–25.
2. Mcmillan DE. The effect of diabetes on blood flow properties. *Diabetes* 1993; **32**: 56–63.
3. Dintenfass L. Blood viscosity factors in severe non-diabetic and diabetic retinopathy. *Biorheology* 1977; **14**: 151–157.
4. Wang B, Wang L, Deng C, Hwang Y, Xie X, Tang N. Abnormality of hemorheology and nail fold microcirculation in diabetes without retinopathy. *Clin Hemorheol* 1991; **11**: 237–242.
5. Schmid-Schonbein H, Volger E. Red cell aggregation and red cell deformability in diabetes. *Diabetes* 1976; **25**: 897–900.
6. Candiloros H, Maller S, Ziegler O, Donner M, Drouin P. Role of albumin glycation on erythrocyte aggregation: an *in vitro* study. *Diabetic Med* 1996; **13**: 646–650.
7. Ernst E, Matrai A. Altered red and white blood cell rheology in type II diabetes. *Diabetes* 1986; **35**: 1412–1415.
8. Pecsvarady Z, Fisher TC, Darwin CH, Fabok A, Maqueda TS, Saad MF, Meiselman HJ. Decreased polymorphonuclear leukocyte deformability in NIDDM. *Diabetes Care* 1994; **17**: 57–63.
9. Chung TW, Liau AU, Yu JJH. Increased red cell rigidity might affect retinal capillary blood flow velocity and oxygen transport efficiency in type II diabetes. *Diabetes Res* 1993; **23**: 75–83.
10. Wolf S, Arend O, Toonen H, Bertram B, Jung F, Reim M. Retinal capillary blood flow measurement with a scanning laser ophthalmoscope. *Ophthalmology* 1991; **98**: 996–1000.
11. Ziopus P, Barbenel JC, Lowe GDO, Macrury S. Foot microcirculation and blood rheology in diabetes. *J Biomed Eng* 1993; **15**: 155–158.
12. Jain SK, Mcvie R, Duett J, Herst JJ. Erythrocyte membrane lipid peroxidation and glycoslyated hemoglobin in diabetes. *Diabetes* 1989; **38**: 1539–1543.
13. Schwartz RS, Madsen JW, Rybicki AC, Nagel RL. Oxidation of spectrin and deformability defects in diabetic erythrocytes. *Diabetes* 1991; **40**: 701–718.
14. Chung TW, Chen TZ, Yu JJH, Lin SY, Chen SC. Effects of α-tocopherol nicotinate on hemorheology and retinal capillary blood flow in female NIDDM with retinopathy. *Clin Hemorheol* 1995; **15**: 775–782.
15. Rampling MW, Gaffney PJ. The sulfate precipitation method for fibrinogen measurement. *Clinica Chimica Acta* 1976; **67**: 43–52.
16. Menard L, Dempsey ME, Blankstein LA, Aleyassine H,

© 1998 John Wiley & Sons, Ltd.

Wacks M, Soeldner JS. Quantitative determination of glycosylated hemoglobin A1 by agar gel electrophoresis. *Clin Chem* 1980; **26**: 1598–1602.
17. Schmaizer EA, Skalak R, Usami S, Vago M, Chien S. Influence of red cell concentration on filtration of blood cell suspensions. *Biorheology* 1983; **20**: 29–40.
18. Chung TW, O'Rear EA. Assessing erythrocyte filterability with 3 μm pore size polycarbonate membrane at constant cell flux. *Clin Hemorheol* 1990; **10**: 505–514.
19. Dintenfass L. Internal viscosity of red cell: problems associated with definition of plasma viscosity and effect volume of red cells in blood viscosity equation. *Biorheology* 1975; **12**: 1480–1486.
20. Stocks J, Dormandy TL. The autoxidation of human red cell lipids induced by hydrogen peroxide. *Br J Haematol* 1971; **20**: 95–111.
21. Jain SK, Levine SV, Duett J, Hollier B. Reduced vitamin E and increased lipofusion products in erythrocytes of diabetes rates. *Diabetes* 1991; **40**: 1241–1244.
22. Koyama T, Araiso T. Effect of α-tocopherol nicotinate administration on microdynamics of phospholipids of erythrocytes in human subjects. *J Nutr Sci Vitaminol* 1988; **34**: 449–457.
23. Berthault MF, Othmane A, Guillou J, Coundord JL, Ktorza A, Dufaux J. Hemorheological abnormalities in rats with experimental mild diabetes: improving effect of troxerutine and α-tocopherol. *Clin Hemorheol* 1994; **14**: 83–92.
24. Reaven PD, Herold DA, Barnett J, Edelman S. Effects of vitamin E on susceptibility of low density lipoprotein and low density lipoprotein subfractions to oxidation and on glycation in NIDDM. *Diabetes Care* 1995; **18**: 807–815.
25. Thurston GB. Rheological parameters for the viscosity, viscoelasticity and thixotropy of blood. *Bioreology* 1979; **16**: 149–162.
26. Isogai Y, Ikemoto S, Kuchiba K, Ogawa J, Yokose T. Abnormal blood viscoelasticity in diabetic microangiopathy. *Clin Hemorheol* 1991; **11**: 175–182.
27. Stoltz JF, Lucius M. Viscoelasticity and thixotropy of human blood. *Biorheology* 1981; **18**: 453–473.
28. Mcmillan DE, Strigberger J, Utterback NG. Rapidly recorverd transient flow resistance: a newly discovered property of blood. *Am J Physiol* 1987; **253**: H919–H926.

© 1998 John Wiley & Sons, Ltd.